

## ENDOCRINE AND METABOLIC CHANGES IN HUMAN AGING

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"Aging and death are inevitable. We have no desire to prolong life, if such prolongation entails only added days and years of misery and suffering. On the other hand, for the first time in history, man has within his grasp potent medications of many kinds, among the more important of which are specific hormones capable of retarding if not stopping some of the undesirable features of growing old".

Thomas Hodge McGavack  
Geriatrics 18:181-191 (1963)

### ABSTRACT

Numerous alterations in hormonal secretion occur with aging. In general, these tend towards a disintegration of the normal cyclic secretory patterns resulting in lower total circulating levels. In addition, declines in receptors and postreceptor function further decreases the ability of the hormonal orchestra to maintain coordinated function throughout the organism. Clues to some of these age-related changes in humans may come from the study of simpler organisms where regulatory systems are known to modulate the aging process. In particular, the interactions among the environment, hormones, and insulin receptor genes have led to new insights into the genetic control of longevity and the development of syndrome X.

It is now well established, as summarized in Table 1, that the levels of many hormones are altered with aging (1). Some hormonal levels decrease, reflecting endocrine gland failure, whereas others increase, often because of end organ failure due to a decrease in receptors or a failure of postreceptor function. In the last decade, it has become popular to look to hormone replacement therapy as a mechanism to reverse the aging process. It has become popular to consider this the "hormonal fountain of youth".

Table 1: Hormonal Alterations with Aging

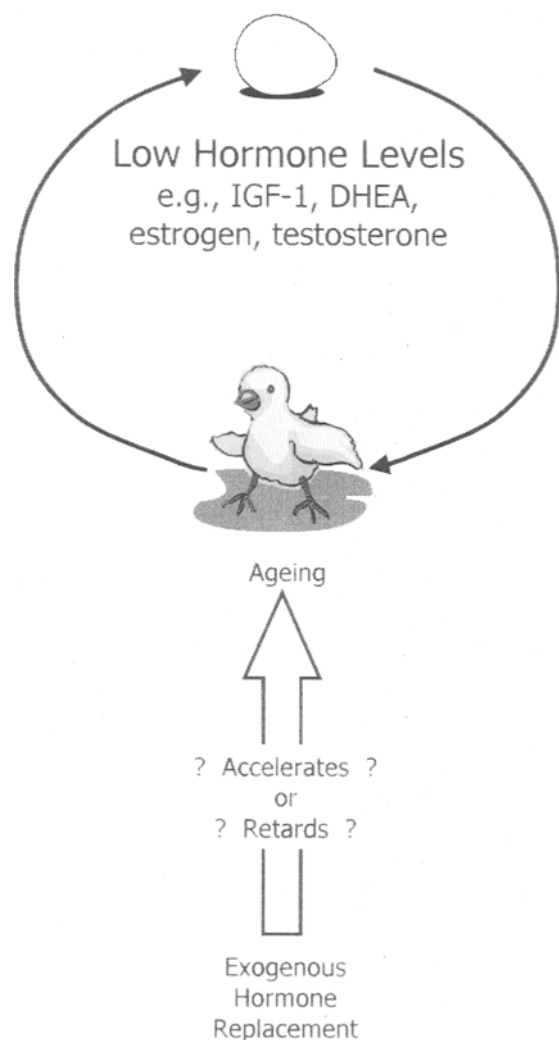
<u>Decrease</u>	<u>No Change</u>	<u>Increase</u>
Growth hormone	Prolactin	Luteinizing Hormone (females)
	Thyrotropin	Follicle Stimulating hormone
Luteinizing Hormone (males)	Thyroid hormones	Cortisol
Insulin Growth Factor I	Epinephrine	Prolactin
Testosterone	Amylin	Norepinephrine
Estradiol		Insulin
Dehydroepiandrosterone		Parathormone
Pregnenolone		Cholecystokinin
25(OH) Vitamin D		
Aldosterone		
Vasoactive Intestinal Peptide (nocturnal surge)		

With aging, dysregulation of food intake accompanied by sarcopenia ('wasting of the flesh') occurs (2). A number of hormonal mechanisms are related to the physiological anorexia of aging (3). In particular, recent studies have suggested that alterations in circulating testosterone levels lead in males to increased leptin levels and thus to anorexia (4).

Another area of intense interest in understanding the mechanisms of aging in humans has been the alterations that occur in glucose metabolism with aging and the role of age-related glucose end products (AGE) in accelerating the aging process (5). This area has been closely linked with the changes in nutrition that occur with aging, with insulin resistance, and more recently, with the genes that encode for the insulin receptor. It is of interest that the hormonal changes produced by dietary restriction in rodents are similar to the age-related hormonal changes in humans, as shown in Table 2 (6). This raises the possibility that these hormonal changes are protective, i.e. slow down metabolism and thus slow down the aging process. This creates a "chicken or egg" hypothesis, illustrated in Figure 1, which asks the question: do aging changes occur because of the decline in hormones or do high hormone levels produce aging changes?

This review will briefly examine the available data in each of these areas and relate the findings to general mammalian theories of aging. Specifically, we will review the literature on the changes in sex steroids, vitamin D, adrenal steroids, and growth hormone. We will then explore symbiotic dysregulation as a theory of aging as exemplified by hormonal dysregulation leading to the anorexia of aging. Finally, we will consider the theories of aging that involve caloric restriction, the *daf*

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**Figure 1:** The chicken and egg hypothesis of the role of hormone replacement in older persons.

genes, and metabolic and oxidative stresses. These latter theories will be explored in the context of syndrome X in the elderly.

### THE HORMONAL FOUNTAIN OF YOUTH

**Estrogen:** Estrogen levels decline over a few years at the time of the menopause. The average age of menopause is 51 years. Menopause is an excellent marker of physiological age; women who have a late natural menopause outlive women who have an earlier menopause (7). In primates, menopause is accompanied by an increase in LH and FSH levels. This is in comparison to other mammalian species, where menopause is triggered by altered hypothalamic behavior. In these species, gonadotrophin levels only increase at extreme old age.

Numerous benefits of hormonal replacement therapy (either estrogen alone or in combination with progesterone) have been demonstrated, as listed in Table 3. Key effects of estrogen on prolonged survival are its

ability to reduce hip fracture (8) and cardiovascular disease (9) by about 50%. The positive cardiovascular effects of estrogen include lowering LDL levels, increasing HDL levels, reducing LDL oxidation (and thus free radical damage), enhancing the synthesis of vasodilating substances (nitric oxide and prostacyclin), decreasing the activity of vasoconstrictors (endothelin 1), and acting as an angiotensin converting enzyme inhibitor (10). Hormone replacement therapy also decreases colon cancer by 30 to 46% (11). Epidemiological studies are inconsistent on whether estrogen decreases the prevalence of Alzheimer's disease (12). However, animal and in vitro studies suggest that estrogen decreases plasma levels of apolipoprotein E4, decreases amyloid secretion, increases neuronal dendrites, enhances the effectiveness of glutamate and acetylcholine on memory, and reverses memory dysfunction in the SAMP8 mouse model of Alzheimer disease (13,14 and unpublished observations).

**Table 2:** Comparison of the effects of aging and dietary restriction on hormone levels (Adapted from reference – No copyright as work done for US Government)

<i>Hormone</i>	<i>Aging</i>	<i>Dietary Restriction</i>
Insulin	Increase	Decrease
Insulin Receptor mRNA	Increase	Increase
Glucose	No change/increase	Decrease/no change
Corticosterone	Increase	Increase
Glucocorticoid Receptor mRNA	Increase	No change
Insulin Growth Factor 1	Decrease	Decrease
IGF1 Binding Protein	Decrease	Decrease
Growth Hormone	Decrease	Decrease
Thyroxine	Decrease	Decrease
Triiodothyronine	Decrease	Decrease
25 (OH) Vitamin D	Decrease	No change
1,25 (OH) Vitamin D	Decrease	No change
Parathormone	Increase	Decrease
Testosterone (Males)	Decrease	Increase
Androgen Receptor mRNA	Decrease	Decrease attenuated
Estradiol (Females)	Decrease	Decrease
Luteinizing Hormone	Decrease	Decrease
Norepinephrine	Increase	No effect
Cholesterol	Increase	No change

**Table 3:** Effects of Hormonal Replacement Therapy in Women (? = not proven)

<i>Beneficial</i>	<i>Disadvantageous</i>
Prevention of osteoporosis and hip fracture	Continued bleeding
Increased HDL	Endometrial cancer
Decreased LDL	? Breast cancer
Increased prostacyclin and nitric oxide	Thrombosis
Decreased Endothelin 1	Thrombophlebitis
Angiotensin Converting Enzyme Inhibitor	
? Enhanced cognition	
Reduced vasomotor symptoms	
Increased resistance to urinary tract infections	
Reversal of atrophic vaginitis	
Decreased Colon Cancer	

The negative effects of estrogen are that it produces an increase in endometrial cancer and is epidemiologi-

cally associated with an increase in breast cancer incidence (10). However, for both of these diseases, the death rates of women developing them while on estrogen are lower than would be expected if they were not (15).

**Testosterone:** Testosterone in men declines with age both in cross-sectional (16) and longitudinal studies, whereas sex hormone binding globulin increases (17). This results in low levels of free and bioavailable (free plus albumin bound) testosterone (18). The decline in testosterone occurs both because of a failure of the testicular leydig cells and a failure of the hypothalamic-pituitary axis (19). In particular, GnRH is released chaotically, in more pulses with less amplitude (20,21). This results in a decreased release of luteinizing hormone from the pituitary (22). Testosterone receptors decline with age in animals (23) and lower free testosterone levels are seen in humans who have less CAG repeats in exon-1 of the androgen receptor (24).

Table 4 summarizes a number of studies demonstrating positive effects of testosterone replacement in men, similar to those seen with estrogen in women (25). In humans, testosterone increases muscle mass (26), muscle strength (27-29), bone mineral density (29,30), hemoglobin (28), and visuospatial cognitive function (31). Studies in the SAMP8 mouse, an animal model of Alzheimer disease (32), suggest that testosterone may play a role in the decline in memory and the overproduction of amyloid- $\beta$  protein seen in this species (14). Surprisingly, testosterone also appears to protect against cardiovascular disease in older humans (33-35). The effects of testosterone on the prostate remain controversial, but appear to be much less negative than originally believed (36). A number of the positive effects of testosterone may be secondary to its ability to increase tissue levels of insulin growth factor-1 (25).

**Table 4:** Effects of Testosterone Replacement in Older Men

1. Increased muscle mass
2. Increased muscle strength
3. Increased bone mineral density
4. Decreased body fat
5. Decreased serum leptin
6. Increased hemoglobin
7. Improved visuospatial cognitive function
8. Increased Insulin Growth Factor-1
9. Coronary artery vasodilation

Overall, the studies suggest that as in females, maintenance of male hormone levels in old age may improve quality of life. Its effects on longevity are yet to be determined.

**Vitamin D:** 25 dihydroxy vitamin D levels decline with age regardless of the amount of sunlight exposure in the ambient geography (37). Vitamin D is essential for both bone integrity and immune function (38). Vitamin D replacement in female nursing home residents decreases hip fracture rates and prolongs life (39).

**Adrenal Steroids:** Both DHEA and DHEA-sulfate decline dramatically with age (40). The physiological role of

DHEA remains uncertain. In rodents, major effects of DHEA have been reported on memory (41) and the immune system (42). In the absence of adrenal insufficiency (43), studies in humans have been controversial, with high dose DHEA (100 mg per day) increasing muscle mass in males (44) but having varied effects on immune function (44) and behavior (45,46).

Pregnenolone, the precursor adrenal hormone, also declines with age (47). It has positive effects on arthritis (48) and appears to have a wide safety margin. In mice, it is a neurosteroid and is the most potent memory enhancing agent yet discovered (49). In humans, it improves sleep (50) and increases attention (51). Sih et al (52) failed to demonstrate effects on strength or memory in a double-blind controlled trial.

**Growth Hormone:** Rudman (53) originally proposed that many of the physiological changes of aging were due to the age-related decline in growth hormone - the so-called "growth hormone menopause". However, growth hormone replacement studies have generally been disappointing, finding small increases in muscle mass and no increase in strength (54), but accompanied by a Pandora's box of side-effects. It has been suggested that fewer side effects will be seen with lower doses, but the poor efficacy of higher doses makes the value of lower doses questionable.

Growth hormone has been used in malnourished elders. Preliminary studies were encouraging (55), but a recent carefully controlled study in malnourished intensive care subjects suggested that growth hormone increased death rates (56).

## HORMONAL AGING AS AN EXAMPLE OF SYMBIOTIC DISINTEGRATION

It is now widely believed that cells are comprised of multiple bacterial components which came together to work in a symbiotic manner during evolution, i.e. the SET (serial endosymbiosis theory) theory of Margulis (57,58). Aging cells have a finite dividing capacity (the Hayflick limit) (59) after which they remain for sometime in a quiescent phase before eventual death. Theories of aging involve both the DNA nuclear material, e.g. DNA damage and repair theories (60) and the effects of organelles, e.g. free radical damage based on mitochondrial activity (61). Nuclear DNA plays a role in coordinating the cytoplasmic organelles and thus maintaining their symbiotic activity. Each of the cytoplasmic organelles contains DNA which itself is subject to random environmental damage.

It is proposed that with aging multiple DNA damage at both the nuclear and the cytoplasmic organelles eventually leads to alterations in the inherent cellular rhythms. The first evidence of this symbiotic disintegration is the inability of the cell to divide followed by more chaotic behaviors leading eventually to the inability of the cell to integrate basic survival patterns and death.

There is now strong evidence that central nervous system neurons develop chaotic secretion of hypotha-

lamic releasing factors during the aging process (62). These changes lead to altered pituitary hormone secretion and eventual changes in the circulating levels of end organ hormones. This altered rhythmicity of hormonal secretion with aging most likely represents the alteration in individual cell rhythms, which occur as a result of symbiotic disintegration. While at present this is obviously only a hypothesis, it is one that unites many disparate theories of aging and is, in fact, imminently testable.

#### ANOREXIA OF AGING: AN EXAMPLE OF HORMONAL DYSREGULATION

Physiological aging is associated with a decline in food intake (63). Males decrease their food intake more than females in both relative and absolute amounts. Like so many aspects of aging, the pathogenetic factors are multiple (63) and include alterations in the hedonic qualities of food (taste and smell), in the satiating effects of food (due to changes in the mechanical and sensory properties of the gastrointestinal tract), in fat cell signaling of total body adiposity, and in the responsiveness of the brain to peripheral signals, [e.g. changes in the central opioid regulation of feeding (64,65)]. Two of these systems are hormonally dependent and will be discussed in greater detail.

When food enters the stomach, the fundus must relax to accommodate the meal (adaptive relaxation) (66). Relaxation is dependent on the elaboration of nitric oxide (67). Evidence in rodents suggests that NO declines in the fundus with aging leading to less adaptive relaxation and more rapid entry of food into the antrum. Antral stretch appears to be the primary factor responsible for the termination of a meal (68). Cholecystokinin (CCK) has direct effects on the antrum and pylorus, slowing the egress of food from the stomach and so indirectly increasing antral stretch. Both basal and fat stimulated CCK levels are increased with aging (69). In addition, CCK appears to more potently stimulate satiation in older animals (70,71). In sum, these changes result in the early satiation commonly seen in older individuals.

At middle age, there is an increase in adiposity in both men and women leading to increases in circulating leptin levels (72,73). Leptin is a peptide hormone that decreases food intake and increases metabolic rate (74). Higher leptin levels in postmenopausal women have been associated with lower food intake (75). Beyond 60 years of age, total body fat declines both in men and women (76,77). This is associated with a decline in leptin levels in women (73). In men, however, decreased testosterone causes an increase in leptin levels despite a decline in total fat mass (4). Testosterone replacement results in leptin levels decreasing (28). This suggests that elevated leptin levels play a role in the greater anorexia developed by males with aging.

The physiological anorexia of aging interacts with disease processes to produce severe anorexia and

malnutrition (78). Cytokines released during disease processes appear to be particularly potent anorectic agents (79). The available data strongly supports a role for hormonal aging in the pathogenesis of age-associated anorexia.

#### SYNDROME X AND THEORIES OF METABOLIC DYSREGULATION

Diabetes mellitus type 2 (DM), especially as manifested in the insulin resistant form of syndrome X (hyperinsulinemia, hyperglycemia, hypertension, hypertriglyceridemia), is a significant cause of morbidity and mortality in the aged and appears to accelerate the aging process (80). This occurs at the genomic level with altered DNA unwinding (81) as well as the protein level with the production of advanced glycation end products (AGE). This section of the review will consider the unique characteristics of the pathophysiology of diabetes mellitus in the elderly and the possible underpinnings of syndrome X. To do this, we will consider the environmental factors that predispose to DM which especially impact on the elderly, the genes affecting insulin resistance and lifespan, and the hormonal interactions which promote the syndrome X phenotype.

##### *Unique Aspects of Diabetes Mellitus in the Elderly:*

DM in the elderly, like diabetes at other ages, is often approached clinically as a lifestyle disease. Obesity secondary to overeating and a sedentary lifestyle results in insulin resistance and even beta cell exhaustion. DM in any age group is more properly considered as an interaction of environmental and genetic factors that impact on the hormonal and neurotransmitter milieu in which the peripheral tissues and central nervous system function and interact. The genetic underpinnings of DM likely include not only the predisposition to insulin dysfunction, but also the predisposition to develop the risk factors, including obesity.

Important work by Arner et al (82) and Meneilly et al. (83) summarized in Table 5 has shown differences between elderly and middle-aged diabetics. All patients with diabetes mellitus, including those with type 1, have lost phase-1 insulin release; that is, the ability to immediately release a bolus of insulin into the blood when challenged with an increase in serum glucose. However, the usual causes of hyperglycemia in middle-aged diabetics are impaired release of insulin and resistance to its actions. This manifests as an impairment in the sustained release of insulin when challenged with an increased serum glucose (phase-2 insulin release), increased glucose output by the liver in the fasting state, an impaired ability of insulin to decrease hepatic glucose output, and an impaired ability of insulin to stimulate glucose uptake into insulin sensitive tissues, such as fat and muscle. In contrast, elderly diabetics have normal hepatic production of glucose in both the fasting state and in response to insulin.

DM is not a monolithic disease in the elderly, however, as shown in Table 5. Lean elderly diabetics likely have

a different mechanism underlying their disease than do the obese elderly (82,83). Lean elderly diabetics, unlike middle aged and obese elderly diabetics, have normal rates of insulin-mediated glucose disposal. DM in the lean elderly is, therefore, primarily a problem of glucose-induced insulin release. DM in the lean elderly resembles diabetes mellitus type 1 in other ways, including the presence of antibodies directed to pancreatic islet cells and an increased frequency of certain haplotypes (84). In comparison, the obese elderly with DM have preserved phase-2 insulin release, consistent with insulin resistance. In an older African American population, there was little difference between the body mass index of the diabetics and nondiabetics (85). In this population, DM and its complications are a major cause of functional deterioration and falls.

**Table 5:** Insulin Actions in Middle Aged, Elderly Lean, and Elderly Obese Patients with Diabetes Mellitus Type 2.

	Middle Aged Diabetics	Elderly Lean Diabetics	Elderly Obese Diabetics
1st Phase Insulin Release	Absent	Absent	Absent
2nd Phase Insulin Release	Decreased	Decreased	Preserved
Hepatic Glucose Release: Fasting	Increased	Normal	Normal
Hepatic Glucose Release with Insulin Infusion	Poorly Suppressed	Normal	Normal
Insulin-Mediated Glucose Disposal	Impaired	Normal	Impaired
Non-Insulin Mediated Glucose Disposal		Severely Impaired	impaired

Classifying elderly diabetics as lean or obese may have important implications for treatment. Meneilly (86) has suggested that lean elderly diabetics respond better to treatments which increase insulin (e.g., insulin injections, sulfonylureas), whereas the obese elderly diabetics respond better to treatments which improve insulin function (e.g., troglitazone, metformin).

The European Group for the Study of Insulin Resistance concluded that aging *per se* is not associated with the development of insulin resistance (87). They found a slight decline with age in insulin action in a group of 1146 men and women aged 18-85 years of age. However, when the results were adjusted for BMI, the relation between age and insulin action was no longer statistically significant.

By comparison, the healthy elderly have a decreased insulin-independent uptake of glucose in the euglycemic, but not the hyperglycemic, state (83). The central nervous system is the major tissue consuming glucose by an insulin-independent pathway and glucose transport across the blood-brain barrier (BBB) is decreased with aging (88). It is unclear whether this decreased transport is due to a primary defect in BBB function or is in response to a decreased metabolic demand from the

brain. Impaired transport of glucose across the BBB has been reported by (89) in a family with seizures and mental retardation and transport is decreased in Alzheimer's disease.

Elderly diabetics have a further decrease of about 20% in the insulin-independent disposal of glucose (90). This could be one of the mechanisms for decreased cognitive function in DM. These patients also have an impaired disposal of glucose even during hyperglycemia, which Meneilly and workers have suggested indicates an impairment in the insulin-independent portion of glucose uptake by skeletal muscle.

The glucagon response to hypoglycemia is impaired in middle aged diabetics, but not the epinephrine, cortisol, or growth hormone responses. In healthy elderly patients without diabetes, the responses of glucagon, epinephrine, and growth hormone to hypoglycemia are impaired and autonomic signs and symptoms such as sweating, palpitations, hunger, and anxiety are decreased. Elderly diabetics have a glucagon response to hypoglycemia that is somewhat more impaired than in healthy elderly, but have a dramatic release of epinephrine, exceeding by about two fold the rise in blood levels seen in the healthy young. Despite the large increase in blood epinephrine levels, the elderly diabetic is less aware of hypoglycemic events. As might be expected from this, the incidence of severe and even fatal hypoglycemia increases dramatically with age.

Elderly patients taking human insulin are less aware of their hypoglycemic episodes than those patients taking pork or beef insulin, despite the fact that there is no difference in counterregulatory hormone response (91). This finding may be consistent with the dual mechanisms by which insulin conveys information about homeostasis to the brain (92). High insulin levels induce hypoglycemia and the resulting decrease in CSF glucose stimulates feeding and other actions designed to normalize blood sugar. Under normal euglycemic conditions, a small fraction of insulin crosses the BBB to enter the CNS. Insulin within the CNS may counter the effects of blood-borne insulin and so insulin may act to some degree as its own counterregulatory hormone by entering the CNS. These counter actions could include attenuating the perception by the brain of the relative severity of the hypoglycemic event. Since pork and beef insulin probably cross the human BBB less well than human insulin, it likely has an attenuated CNS insulin action and, therefore, hypoglycemia is more robustly appreciated.

The obese, elderly diabetic is most likely to suffer from insulin resistance. We will now consider some of the environmental, genetic, and hormonal interactions which can lead to insulin resistance.

**Environmental Factors Impacting on the Elderly Diabetic:** Several factors combine to put the elderly at increased risk for DM. Poor nutrition can lead to decreases in vitamins and trace minerals. Decreases in potassium and magnesium, for example, impair insulin

action and energy usage via the sodium-potassium-magnesium ATPase pump.

Decreases in antioxidants such as vitamins C and E may also impair insulin action and, as described below, insulin resistance is related to oxidative stress in other ways. Diets low in complex carbohydrates and high in saturated fats and sugars can increase the incidence of glucose intolerance. Altered intake of fatty acids may lead to changes in membrane fluidity which, in turn, alter receptor function, including the function of the insulin receptor (93). Decreased physical activity and redistribution of body fat and fat/muscle ratios are also associated with the development of glucose intolerance in the elderly. Polypharmacy, so common in the elderly, can lead to an increased risk of taking a drug interfering with glucose homeostasis.

*Insulin Resistance, daf Genes, and Life Span:* DM in the elderly, as in the middle-aged, has a strong genetic component. DM clusters in families and in some ethnic groups. An identical twin to a patient with DM is more likely to develop DM or to have abnormalities suggestive of early insulin resistance. Identifying the genes responsible for DM or insulin resistance is an area of active research, with much interesting work being done with the nematode *Caenorhabditis elegans*.

In Frank Herbert's Dune, excrement from a worm led to an increased life span. More recently, the genes of a much smaller worm have lent spice to the area of longevity research. Mutations can quadruple the life span of *C. elegans*. Many of the mutations that lead to increased life span occur in genes belonging to the *age*, *clk* (clock), and *daf* families (94). The *daf* genes are developmental genes so named because they control the ability of the nematode to enter into a state of resistance to harsh environmental conditions (the dauer stage) when starvation, high temperatures, or overcrowding occur. The *daf* mutations, therefore, demonstrate genetically controlled links among starvation, metabolism, and life span.

The first clues as to how *daf* mutations could increase life span came when it was discovered that *daf-2* encodes for a protein whose mammalian homologues are members of the insulin receptor family (the insulin receptor, the insulin-like growth factor-1 receptor, and the insulin receptor-related receptor). The *daf* protein is part of a cascade activated when an insulin-like molecule binds to its cell surface receptor. One of the mutations that produces an inactivation of *daf-2* in *C. elegans* leads to insulin resistance and obesity in humans (95). The *daf-2* protein, in turn, activates phosphatidylinositol 3-kinase; the catalytic subunit of this kinase is the homologue of the *age-1* gene. Therefore, the *daf* and *age* mutations are intimately involved in insulin-initiated signaling pathways.

The connection provided by *daf-2* between insulin receptors and life span has suggested that the *daf* cascade might be used to explore insulin resistance in humans. Since the increased mortality of diabetics is

related primarily to macrovascular disease and complications of microvascular disease, it might be speculated that the *daf* genes are involved in these processes. The *daf-2* mutation in the nematode is associated with increased adiposity and glycogen production, and some of these factors are also altered in diabetes mellitus. However, certain fundamental differences occur between *daf-2* mutations and the typical insulin resistance seen in humans. Specifically, insulin resistance in humans 1) is associated with decreased, not increased, life expectancy; 2) is thought to be the consequence, not the cause, of obesity; 3) leads to decreased, not increased glycogen storage; and 4) results in increased lipolysis rather than fat accumulation.

#### *Insulin Resistance and Longevity: Beyond Glucose:*

To better understand how insulin resistance could affect lifespan, one must recall that insulin does much more than simply stimulate the uptake of glucose by fat and muscle. Insulin is a key regulatory molecule that has direct effects on the CNS, activates intracellular signaling cascades, has mitogenic activity, and regulates the transcription and translation of numerous metabolic genes. Similarly, insulin-like growth factor is involved in various aspects of metabolic control.

One of the exciting aspects of work with *C. elegans* is that it allows study of the complex functions of insulin-like molecules in a more simplified form. The primary function in *C. elegans* of wild-type *daf-2* and *age-1* gene products is to inactivate by phosphorylation the products of the *daf-16* gene (96,97). Null mutations of the *daf-16* gene block the increase in life span induced by *daf-2* and *age-1* mutations. Therefore, the effects of *daf-2* and *age* mutations on lifespan are mediated through *daf-16*. The *daf-16* gene encodes for a forkhead transcription factor, which regulates the activity of numerous genes. The specific genes are currently unknown, but *daf-2* mutants have increased levels of catalase, superoxide dismutase, isocitrate dehydrogenase, isocitrate lyase, malate synthase and lower levels of acid phosphatase and alkaline phosphatase (98), demonstrating that a large number of enzymatic proteins are likely affected.

These mutations are being used to explore some of the classic theories of aging. Glucose utilization/metabolic rate, food deprivation, and free radical scavenging have all been implicated in affecting lifespan in mammals and advocates for those mechanisms have found reflections of their theories in the mutations of *C. elegans*.

A link between metabolic rate and life span was first proposed by Rubner in 1908, who compared six mammalian species ranging in size from a horse to a guinea pig and found an inverse relation between caloric consumption/kg of body weight and life span. These findings give rise to the concept of a metabolic penalty. Reduction of calorie intake has been shown to increase life span by about 30% in numerous species of mammals. Some have argued that the increase in life span from caloric restriction reflects an attenuation of a host of risk factors which arise from the overfeeding found in

captive animals and Western man, such as obesity, hypertension, diabetes mellitus, and hyperlipidemias. Others have argued that increased lifespan with caloric restriction points to a more fundamental role of cellular metabolism in controlling life span. Induction of the *daf* genes with starvation and temperature alterations, the same genes that can affect lifespan, lends support to the metabolic penalty hypothesis. However, it may be that starvation and temperature are only related by way of the dauer phase, a developmental rather than an aging phenomenon, and not involved in a postulated sub-routine of the dauer program which alters lifespan. The adult nematode with a *daf-2* mutation lays down more fat (95), not less as would be consistent with a pathway imitative of starvation. Studies of nematodes with *eat* gene mutations strongly argue against the hypothesis that *daf-2* mutations increase lifespan through a mechanism related to starvation (99). *Eat* mutants are partially starved because of defects in their feeding organ and live longer than wild-type nematodes. The *eat* gene mutation is independent of *daf-16* activity and the double mutant of *daf-2* and *eat* lives longer than the *daf-2* only mutant. These findings show that starvation due to the *eat* mutation is mediated through a pathway separate from that of the *daf* genes.

Oxidative stress is associated with insulin resistance. Free radical levels in the plasma of fasting individuals increase with age and both hyperglycemia and hyperinsulinemia increase oxidative stress. Autooxidation of glucose leads directly to free radical formation and hyperinsulinemia leads to increases in hydrogen peroxide that are not mediated through the receptor kinase step (100). Antioxidants such as vitamin E and lipoic acid can reduce levels of plasma free radicals in blood and improve insulin-mediated glucose uptake and plasma lipid patterns. Lipoic acid may work in part, however, by altering membrane receptor number and function by changing membrane fluidity. Oxidative stress is associated with reduced transport to the membrane surface of the GLUT-4 transporter (the insulin-sensitive glucose transporter). However, it is unclear whether insulin resistance is the cause or the result of increased free radical formation.

Work with *C. elegans* and its *daf* genes has been used to explore this issue as well. High levels of oxygen scavengers, such as catalases and superoxide dismutases (SOD), can rid the cell of reactive oxygen species. In nematodes which no longer synthesize cytoplasmic catalase, the *daf-2* mutation no longer increases life span (101). Several experiments show a good correlation between Mn-SOD activity and life span-related mutations (102). The *daf-16* mutation blocks the *daf-2* associated increase in life span, *sod-3* mRNA, and Mn-SOD activity. The life span of several *daf* mutants and combined mutants correlates with their level of protein carbonyl, an index of oxidative protein damage (103). A study yet to be conducted is to determine the effect of exogenous anti-oxidants on the ability to extend life span in *daf-2* mutants. The difference between the

life span of wild-type and *daf-2* mutant nematodes would be reduced to the extent that *daf-2* mutations act by scavenging radical oxygen species.

**Syndrome X: Hormones Other than Insulin:** Whereas the above focuses on the extra-glycemic actions of insulin, other work has focused on the role of hormones other than insulin in the pathophysiology of syndrome X. Two of these hormones, leptin and tumor necrosis factor- $\alpha$  (TNF), are secreted by fat. Leptin is a 16 kD molecule that crosses the BBB to alter feeding and thermogenesis (104-108). Obesity in humans results from a relative resistance to leptin due to either an impaired ability of leptin to cross the BBB or an impaired action of leptin at its CNS receptors. In both humans and outbred mice, a subpopulation steadily increases their adiposity as they age. We have recently shown that in mice, this subpopulation has an impaired ability to transport leptin across the BBB (109). Therefore, the obesity of maturity and the resulting insulin resistance may be due to an impaired ability of the BBB to synthesize leptin transporters.

TNF has been suggested to play a role in the insulin resistance seen in DM, obesity, infection, and cancer. Correlations exist between age and serum levels of TNF and, independently of age, between serum levels of TNF and leptin (110, 111). Whole body glucose disposal also correlates with TNF levels (111). Elderly men with insulin resistant DM have elevated levels of serum TNF in comparison to healthy elderly men (112). Serum levels of TNF also correlated with BMI, fasting glucose levels, and serum triglyceride levels and inversely with HDL cholesterol levels. In a group of Native Canadians, Zinman et al (113) found that levels of TNF in the serum correlated with insulin resistance, systolic blood pressure, waist circumference, and triglyceride levels. The level of TNF mRNA and its receptors is higher in biopsies of adipose tissue from obese women than from biopsies of non-obese women (114). The levels of the TNF p75 receptor and TNF mRNA from those biopsies correlated with serum insulin and triglyceride levels, whereas the TNF p55 receptor correlated with BMI and fat cell size.

TNF infusions in rats impair the ability of insulin to suppress hepatic glucose production and to stimulate peripheral glucose utilization (115). Obese mice lacking TNF have improved insulin sensitivity, lower levels of free fatty acids (FFA), and increased insulin-stimulated tyrosine phosphorylation of the insulin receptor in muscle and adipose tissue (116). Obese mice without the TNF p55 receptor do not develop insulin resistance (117). Obese mice mutated to express a TNF inhibitor directed at the p55 receptor have increased insulin sensitivity and normal insulin suppression of hepatic glucose output without changes in insulin number or affinity in comparison to non-mutated obese mice (118).

TNF induces insulin resistance by acting through post-receptor pathways. The post-receptor site affected by TNF varies among tissues and even for a given tissue derived from different sources. Several studies have

shown that the thiazolidinediones can reverse some of the insulin resistant effects of TNF. For example, troglitazone reversed the TNF-induced impairment in insulin-stimulated glucose disposal and partially reversed the hyperlipidemia, but had no effect on the increase in insulin levels or on insulin receptor tyrosine kinase activity (119). Interestingly, pioglitazone is able to partially reverse the overexpression of TNF and its receptors seen in muscle and fat from diabetic mice (120).

TNF may act directly or by stimulating the release of other compounds. TNF has been shown to stimulate the release of nitric oxide, FFA, reactive oxygen species, interleukin-1 $\alpha$ , and TGF- $\beta$  and enhance Mn-SOD activity. Each of these substances, in turn, has been implicated in insulin resistance. For example, inhibitors of interleukin-1 and of nitric oxide (NO) block TNF-induced insulin resistance in pancreatic beta cells (121).

Unraveling the interactions among TNF, NO, insulin, and endothelial cell function could reveal the link between insulin resistance and hypertension. Serum levels of endothelin-1, taken as an index of endothelial dysfunction, C-peptide, TNF, and BMI correlate with one another in non-diabetics with android-type obesity, but not in persons with gynecoid type obesity (122). TNF induces NOS and NO release from endothelial cells. Blockade of NO synthesis leads to increased blood pressures in fat, but not lean, rats (123). One study has shown that TNF toxicity to aortic endothelial cells is mediated through NO, whereas another has suggested that NO can protect endothelial cells from TNF (124,125). Insulin also stimulates NO release from endothelial cells, but this effect is lost in insulin resistant subjects (126). This could lead to impaired dilatation with insulin resistance, explaining the hypertensive aspect of syndrome X.

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